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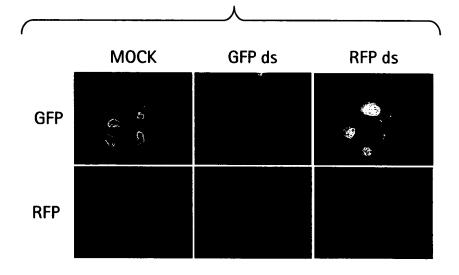
AND STABLE siRNA

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FIG. 1A

Sense strand (ss) 5' GCAGCACGACUUCUUCAAGdTdT GFP ds siRNA Antisense strand (as) dTdTCGUCGUGCUGAAGAAGUUC 5' GFP mRNA target site sequence AAGCAGCACGACUUCUUCAAG 238 2<u>5</u>8 **GFP mRNA** m7GpppG Poly A RFP mRNA m7GpppG Poly A RFP mRNA target site sequence AAGUGGGAGCGCGUGAUGAAC 277 297 Sense strand (ss) 5' GUGGGAGCGCGUGAUGAACdTdT RFP ds siRNA Antisense strand (as) dTdTCACCCUCGCGCACUACUUG 5'

FIG. 1B



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FIG. 1C

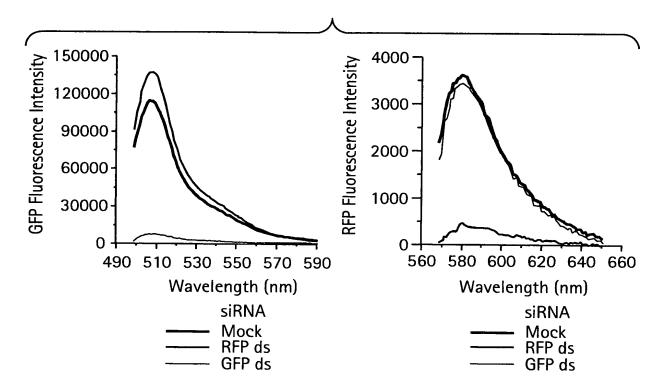


FIG. 1D 1.0 Normalized GFP/RFP 8.0 0.6 0.4 0.2 0.0 6 18 30 42 54 66 **Hours Post Transfection** ☐ GFP as ■ GFP ds

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FIG. 1E

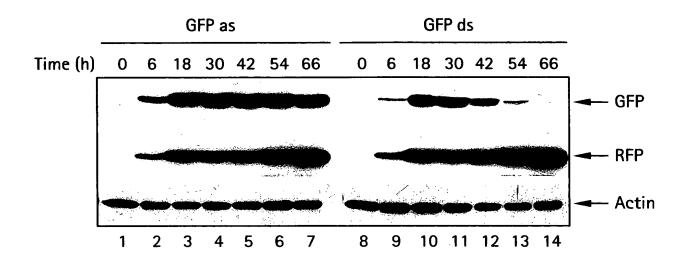
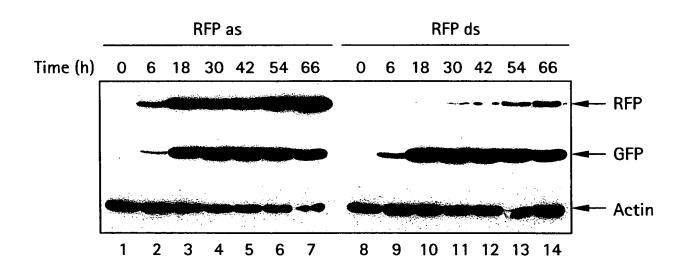
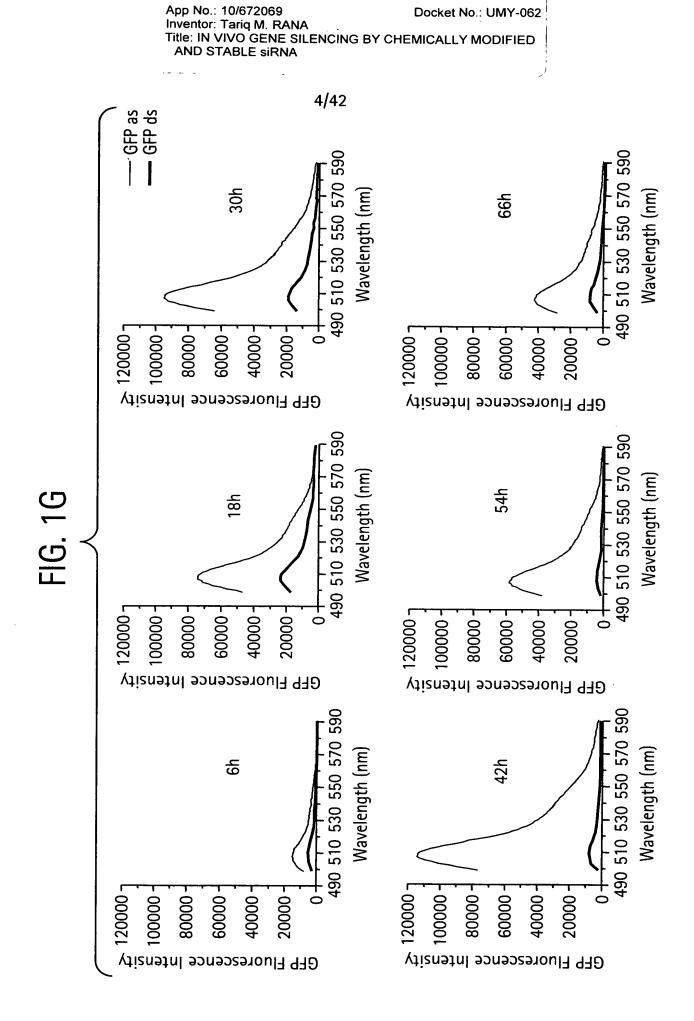


FIG. 1F





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FIG. 2A

5'- Amino modification = N3

$$A_2$$
 Meo

 A_3 Puromycin = Pmn

 A_2 Meo

 A_4 Meo

 A_4 Meo

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FIG. 2B

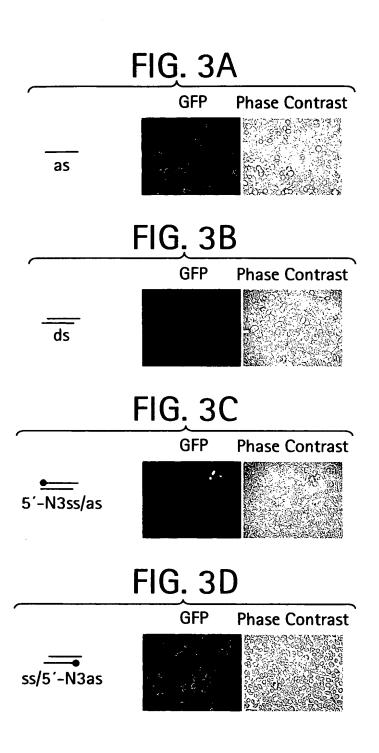
GFP siRNA

	ds (WT)	GCAGCACGACUUCUUCAAGdTdT dTdTCGUCGUGCUGAAGAAGUUC
5' Modified siRNA	5'-N3ss/as	N3 - GCAGCACGACUUCUUCAAGdTdT dTdTCGUCGUGCUGAAGAAGUUC
	ss/5′-N3as	GCAGCACGACUUCUUCAAGdTdT dTdTCGUCGUGCUGAAGAAGUUC- N3
	5'-N3ss/5'-N3as	N3 -GCAGCACGACUUCUUCAAGdTdT dTdTCGUCGUGCUGAAGAAGUUC- N3
3' Modified siRNA	ss3'-Pmn/as	GCAGCACGACUUCUUCAAGdTdT- Pmn dTdTCGUCGUGCUGAAGAAGUUC
	ss/as3'-Pmn	GCAGCACGACUUCUUCAAGdTdT Pmn-dTdTCGUCGUGCUGAAGAAGUUC
	ss3'-Pmn/as3'-Pmn	GCAGCACGACUUCUUCAAGdTdT- Pmn Pmn -dTdTCGUCGUGCUGAAGAAGUUC
	ss/as3'-Biotin Bi	GCAGCACGACUUCUUCAAGdTdT otin-dTdTCGUCGUGCUGAAGAAGUUC
Bulge-containing siRNA	ss-bulge/as	UG GCAGCACGA CUUCUUCAAGdTdT dTdTCGUCGUGCU GAAGAAGUUC
	ss/as-bulge	GCAGCACGA CUUCUUCAAGdTdT dTdTCGUCGUGCU GAAGAAGUUC CA
	ss-bulge/as-bulge	UG GCAGCACGA CUUCUUCAAGdTdT dTdTCGUCGUGCU GAAGAAGUUC CA

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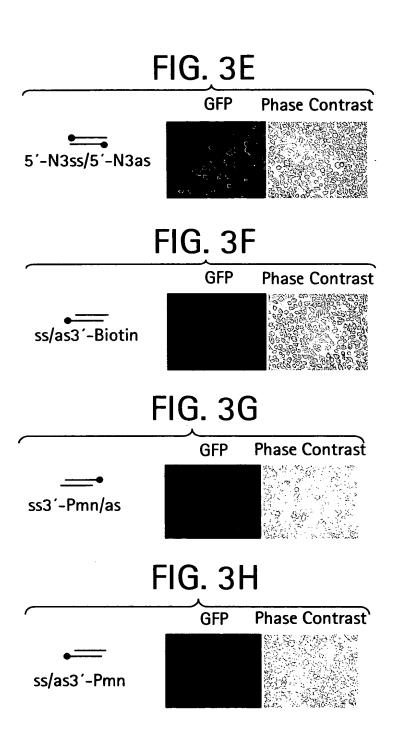


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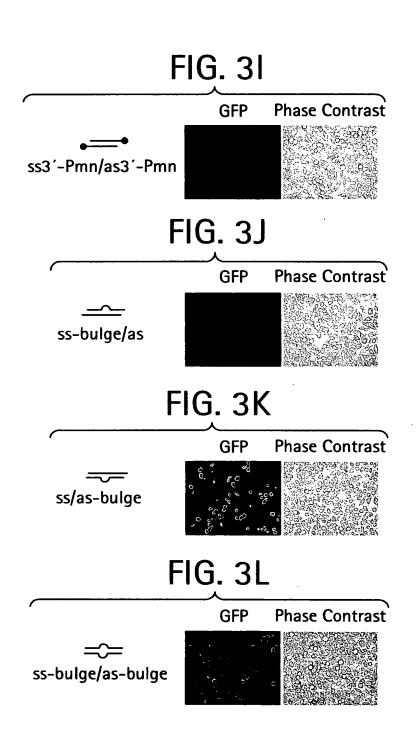


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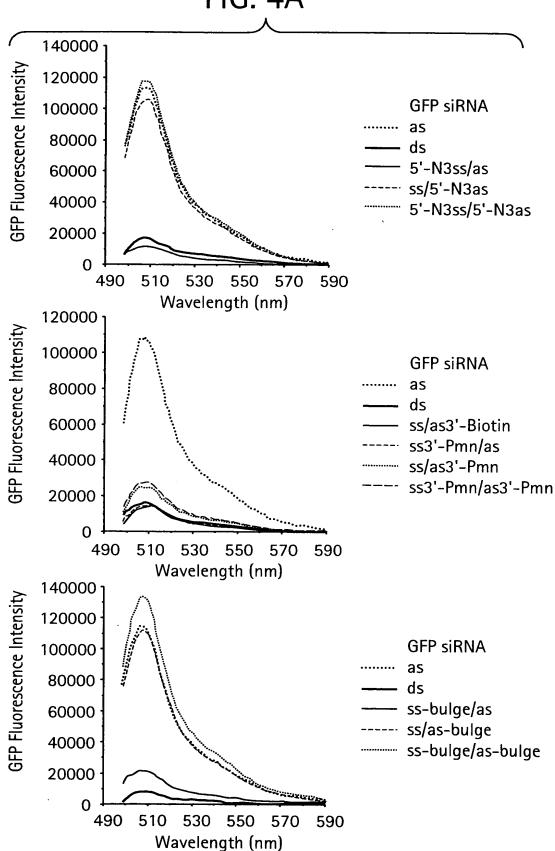
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FIG. 4A

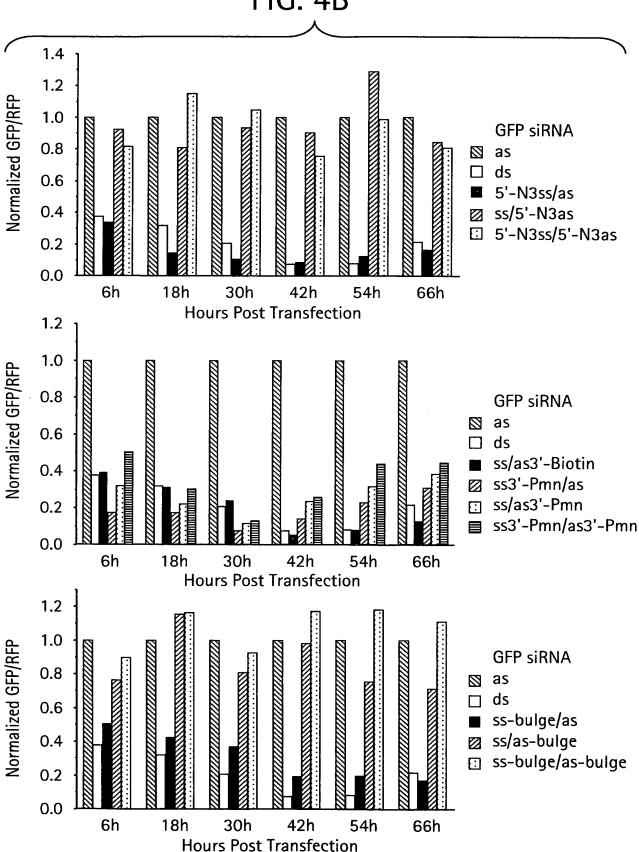


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FIG. 4B

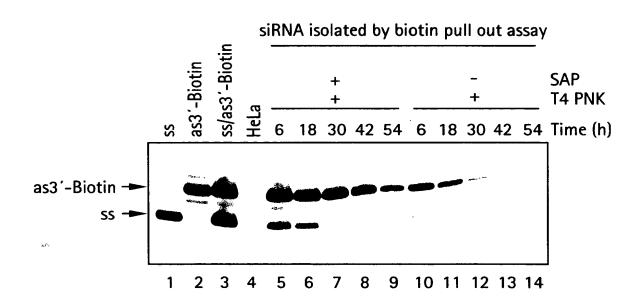


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FIG. 5



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FIG. 6A

Psoralen (HMT)

FIG. 6B

GFP ds siRNA

GCAGCACGACUUCUUCAAGdTdT

dtdtcg**u**cg**u**gcugaagaag**u**uc

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FIG. 6C

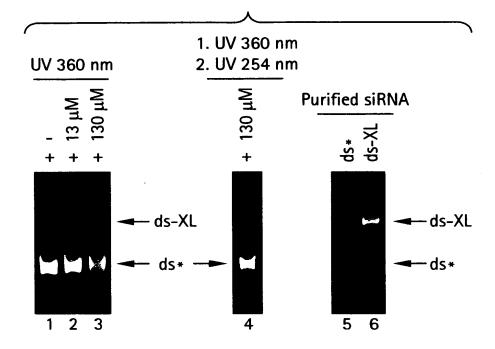
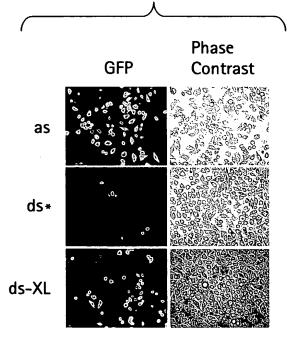


FIG. 6D

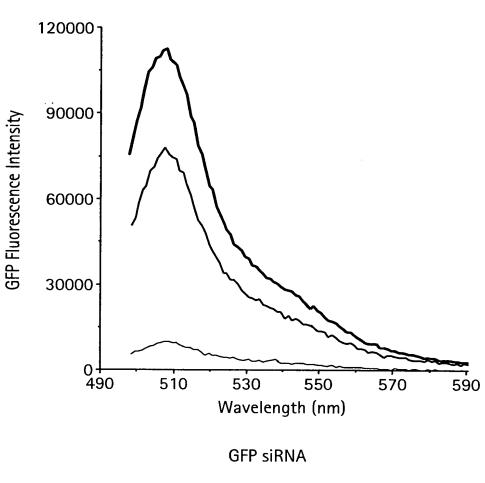


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FIG. 6E



• as

- ds-XL

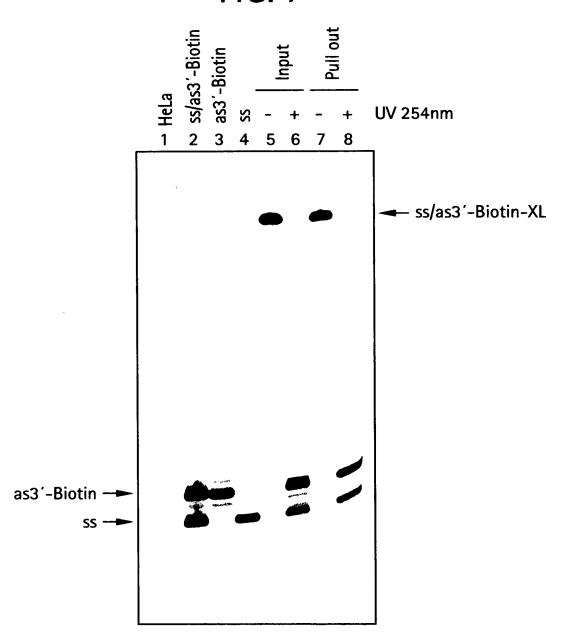
ds*

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FIG. 7



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FIG. 8A

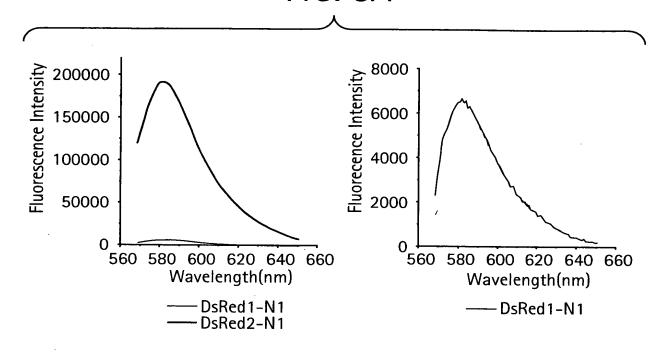
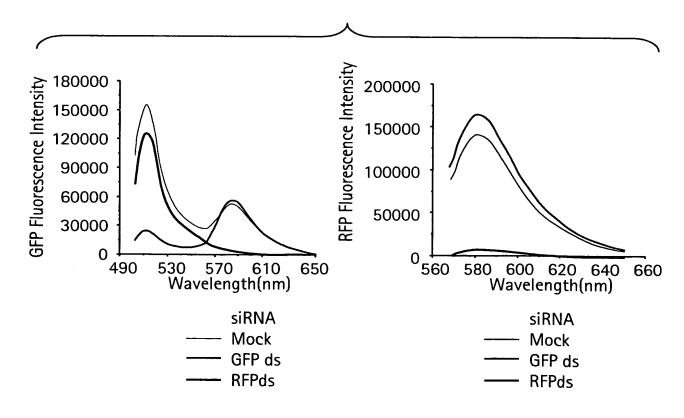


FIG. 8B

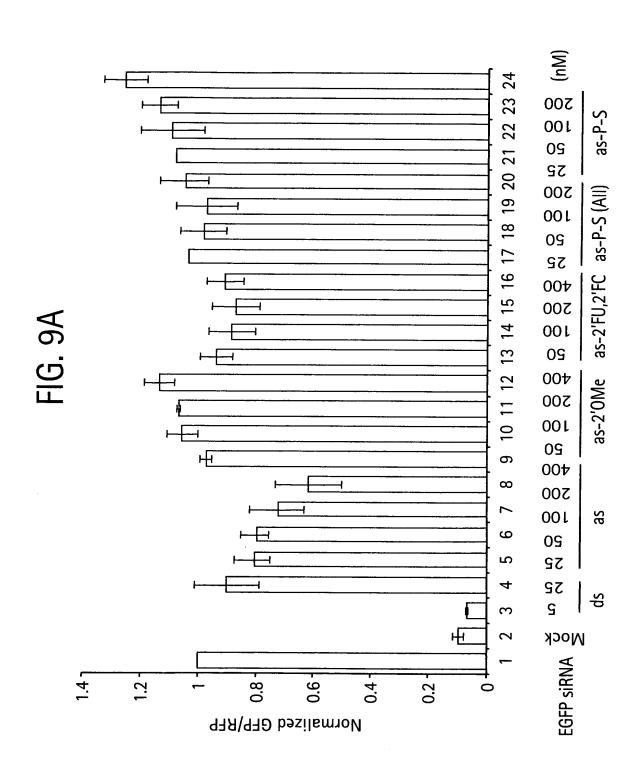


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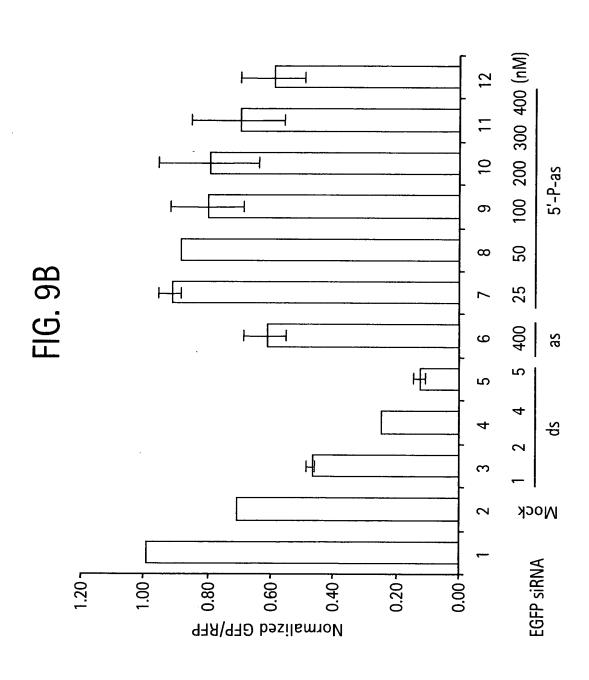


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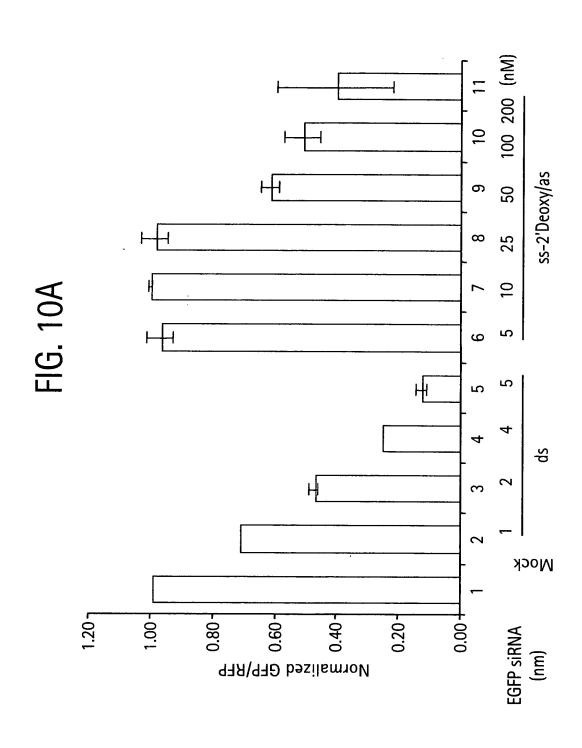


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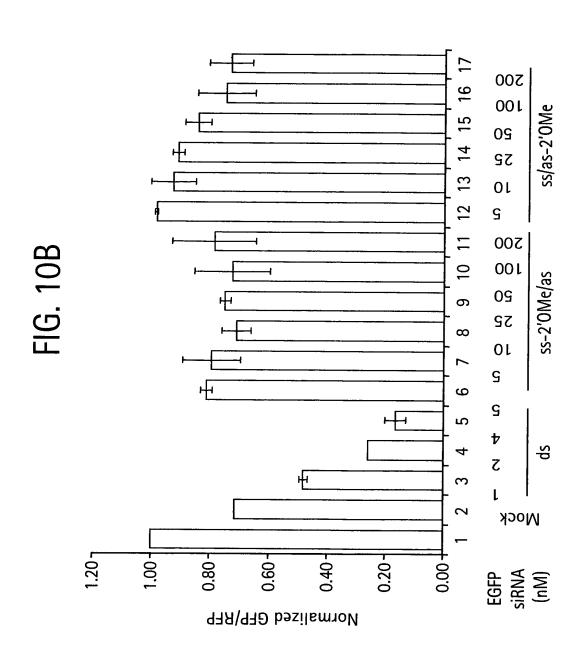


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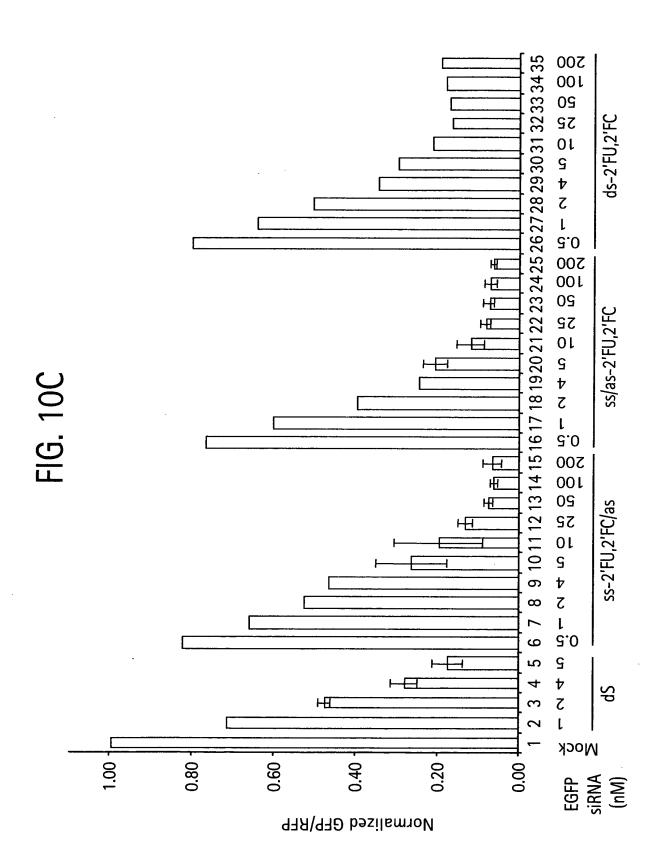
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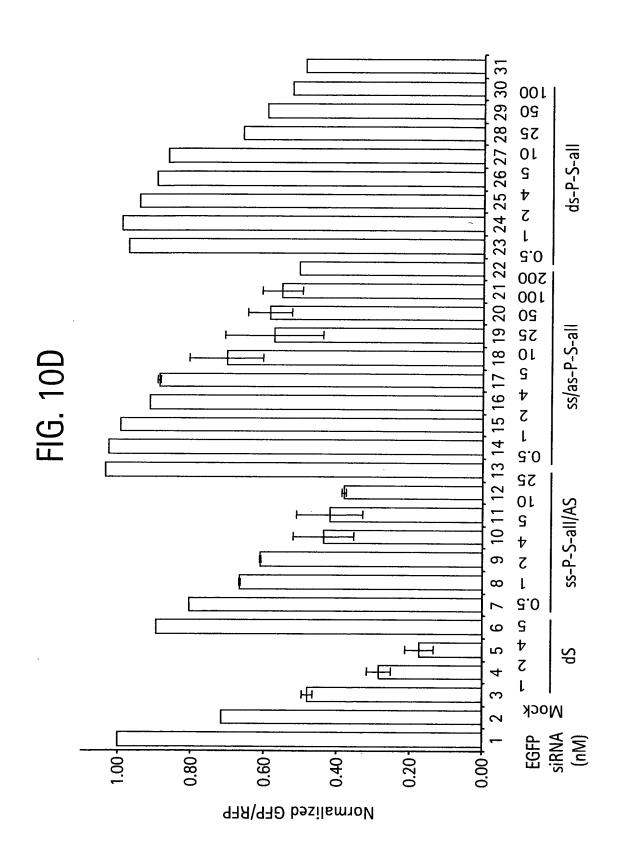
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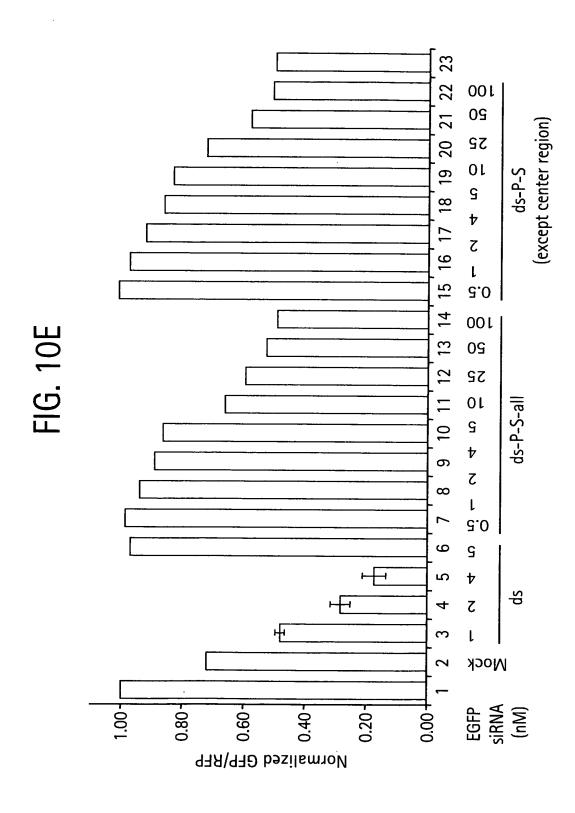
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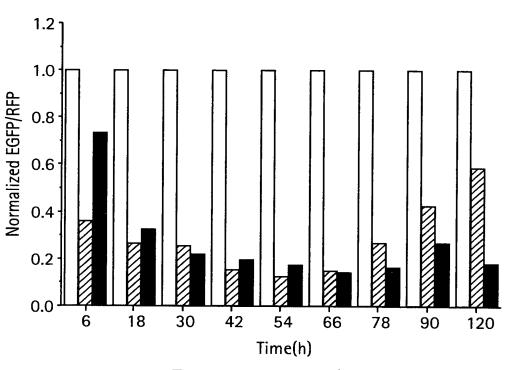


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FIG. 11



- ☐ Mock ☑ EGFP ds EGFP ds-2'FU,2'FC

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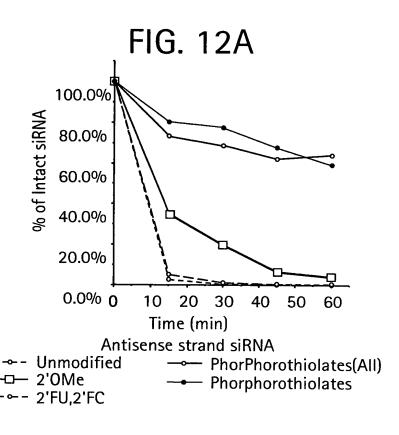
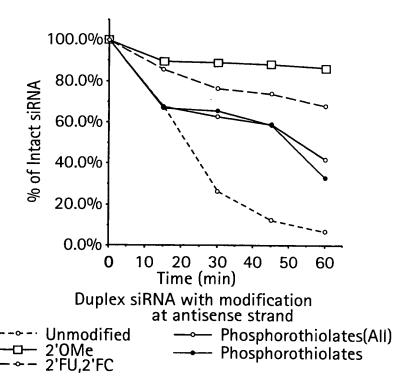


FIG. 12B



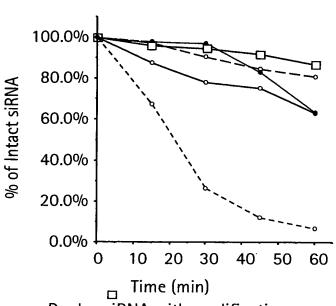
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FIG. 12C



Duplex siRNA with modification at both strand

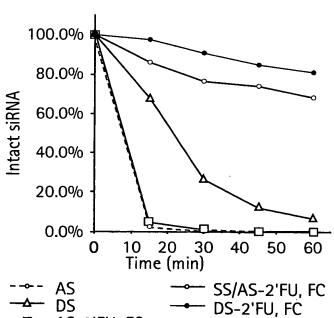
--- Unmodified

-~- 2'FU,2'FC

── PhorPhorothiolates(All)

- Phorphorothiolates

FIG. 12D

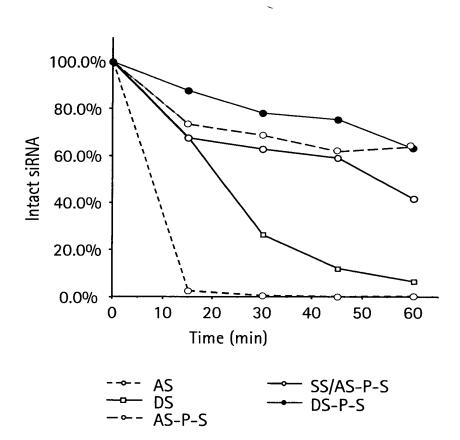


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FIG. 12E



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5' GCAGCACGACUUCUUCAAGATAT dTdTCGUCGUGAAGAAGUUC 5'

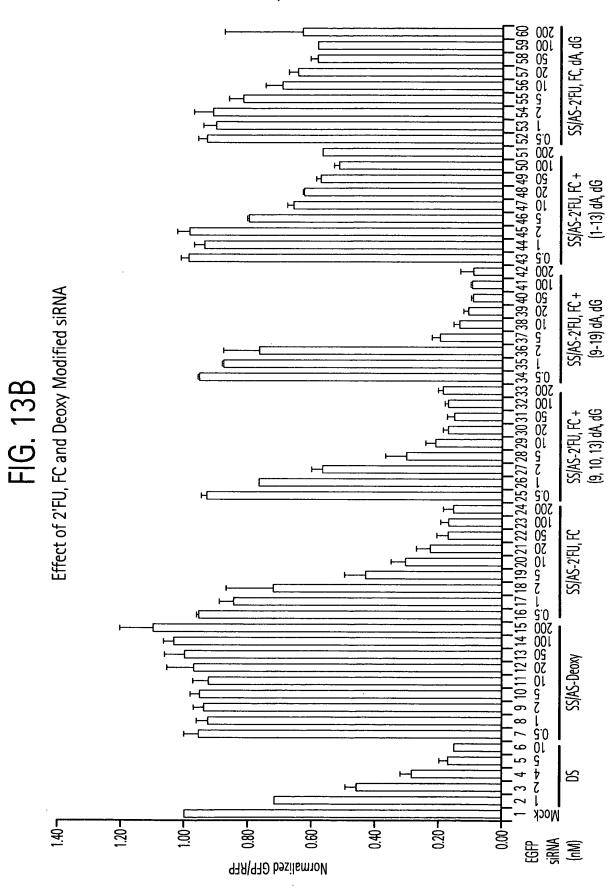
2' structure ■ -0H ■ -H WT DS siRNA

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31/42 SS-2'FU, FC/AS-2'FU, FC + (1-13) dA, dG 5' GCAGCACGACUUCUUCAAGdIdI drdrcgucgucgucaagaaguuc 5' 5' GCAGCACGACUUCUUCAAGdIdI drdrcgucguccucaagaaguuc 5' SS-2'FU, FC/AS-2'FU, FC + dA, dG Poly A |SS-2'FU, FC/AS-2'FU, FC + (9, 10, 13) dA, dG 5' GCAGCACGACUUCUUCAAGdIdI drdrcgucgugcugaagaaguuc 5' SS-2'FU, FC/AS-2'FU, FC + (9-19) dA, dG 5' GCAGCACGACUUCUUCAAGATAT drdrcgucgugcugaagaaguuc 5' AAGCAGGACUUCUUCAAG-Cleavage on mRNA Target EGFP mRNA Antisense strand 5' GCAGCACGACUUCUUCAAGAITAT Sense strand drdrcgucguccugaagaaguuc 5' SS-2'FU, FC/AS-2'FU, FC m7GpppG 5' GCAGCACGACUUCUUCAAGdIdI drdrcgucgugcugaagaaguuc 5' SS/AS-Deoxy siRNA 5' GCAGCACGACUUCUUCAAGdIdI drdrcgucgugcugaagaaguuc 5' WT DS siRNA 2' structure 후 푸 4

FIG. 13C

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AND STABLE siRNA 32/42 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 AS-2'FU, FC, dA, dG SS-2'FU, FC/ SS-2'FU, FC/AS-2'FU, FC Effect of 2'FU, FC and Deoxy Modified siRNA (continued) SS-2'FU, FC/AS-2'FU, FC + (9, 19) dA, dG FIG. 13D SS-2'FU, FC/AS-2'FU, FC + (9, 10, 13) dA, dG SS-2'FU, FC/ AS-2'FU, FC 9 \simeq 1.46 1.20-1.83<u> —</u> 080 0.40 0.20 000 0.60 SiRNA (nM)

Normalized GFP/RFP

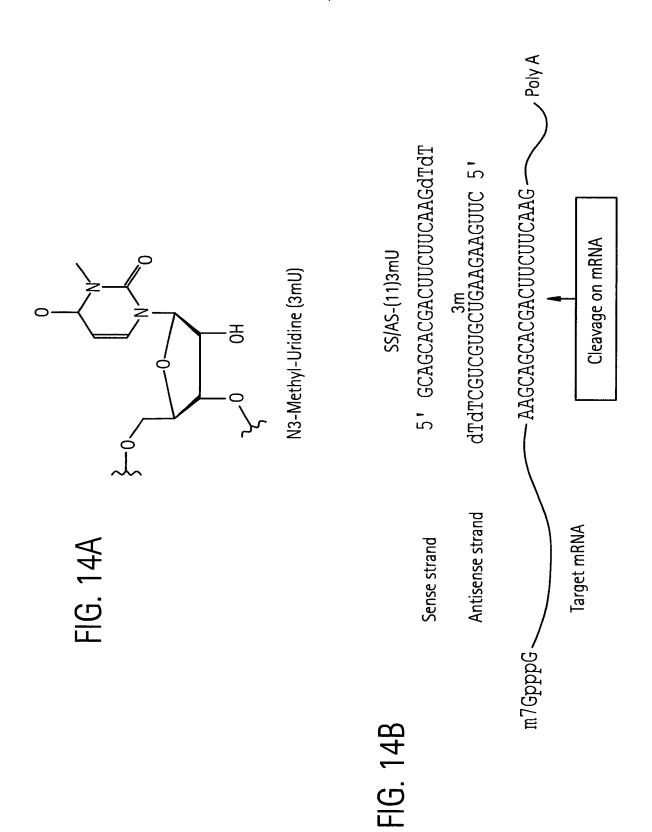
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AND STABLE SIRNA

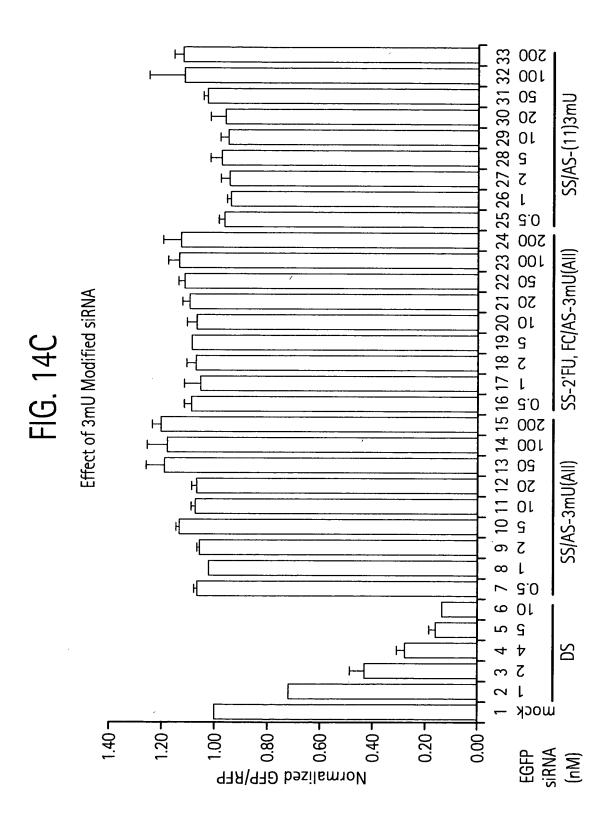
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Docket No.: UMY-062 AND STABLE siRNA 35/42 GCAGCACGACUUCUUCAAGGITGI dTdTcGUCGUGCUGAAGAAGUCU SS/AS-(1, 2)mm GCAGCACGACUUCUUCAAGdIdI GCAGCACGACUUCUUCAAGGTGT drdrgcucgugcugaagaaguuc drdrceucgugcugaagaaguuc SS/AS-(18, 19)mm FIG. 15B

ds

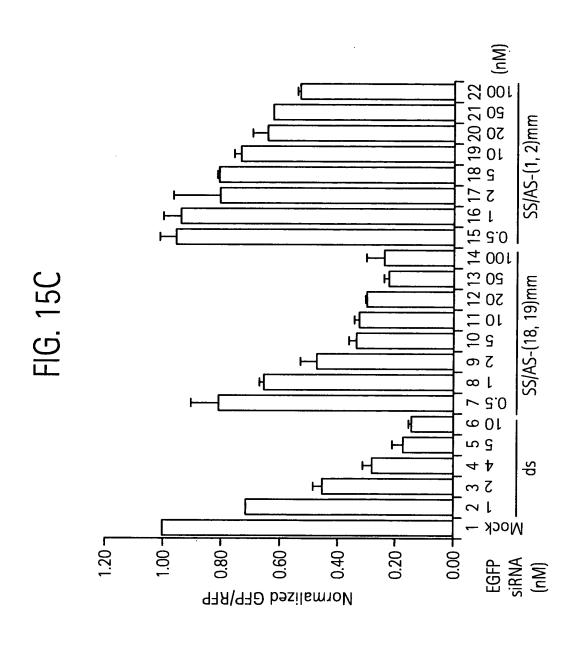
drdrcgucgucgucaagaaguuc

GCAGCACGACUUCUUCAAGGITGI

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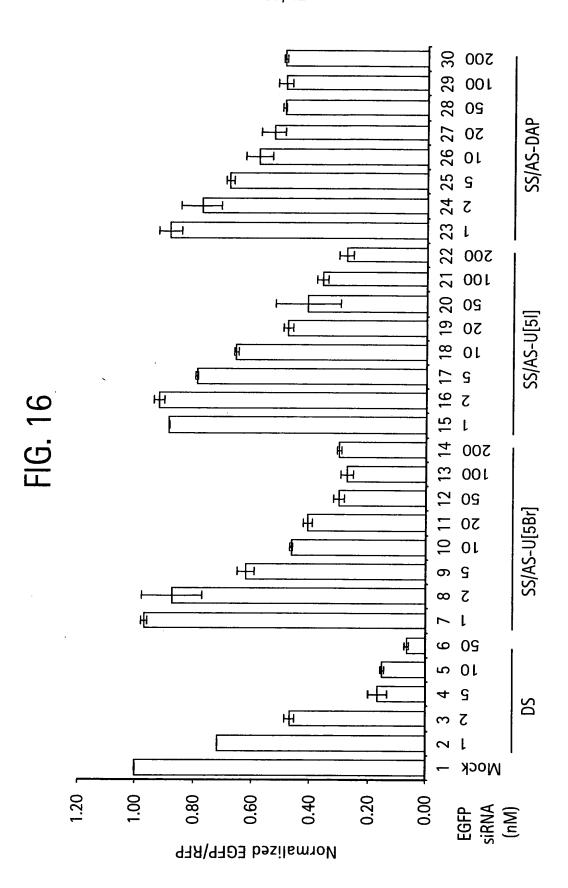


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FIG. 17

SS/AS-2'-FU, FC SS/AS-2'-FU, FC + (9, 10, 13) dA, dG SS/AS-2'-FU, FC + dA, dG SS/AS-2'-FU, FC + (1-13) dA, dG SS/AS-2'-FU, FC + (9-19) dA, dG SS/AS-(1, 2) mm 2 3 5 8 9 10 11 12 13 14 15 16 17

³²P-cap labeled Target **Cleavage Product** ·× ... 38 86. and: os Sig

RISC * Turn-over

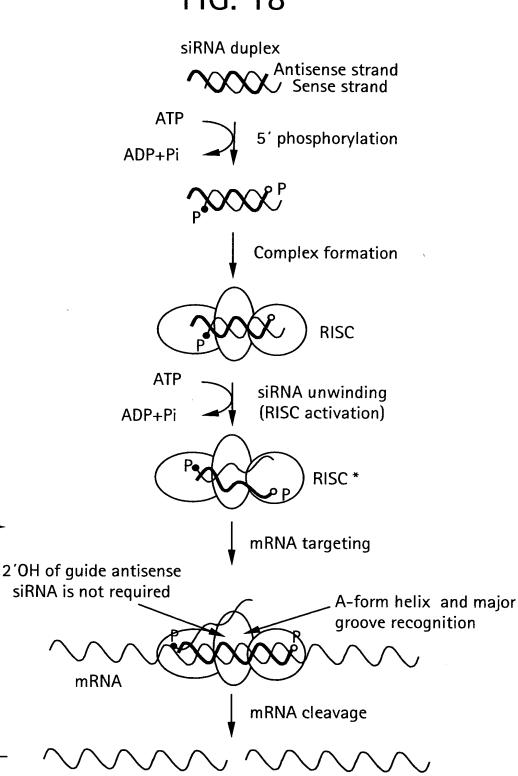
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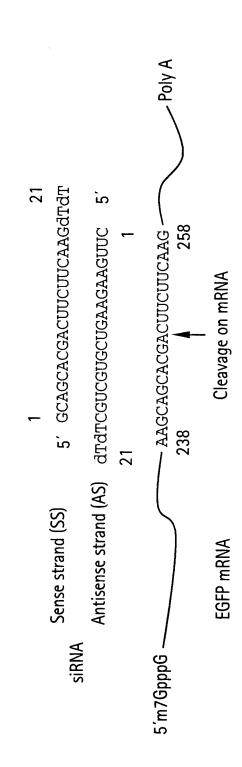
FIG. 18



Degraded mRNA

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FIG. 19B

2'-Fluoro-uridine = 2'FU

 N^3 -Methyl Uridine = 3MU

5-Bromo-uridine = U[5Br]

5-lodo-uridine = U[51]

2,6-Diaminopurine = DAP

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FIG. 20

Photocleavable Biotin